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Regeneration and somaclonal variation in apomictic *Paspalum dilatatum* Poir.

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Summary

In an attempt to incorporate variation into a uniform obligate apomict, plants of apomictic common dallisgrass, *Paspalum dilatatum* Poir., were regenerated from callus derived from immature inflorescences. Plants developed through both organogenesis and embryogenesis. A total of 682 regenerants were produced and more than 400 were transplanted into a field nursery and screened for somaclonal variation. Eventually 20 regenerants were selected, increased, and planted into a replicated nursery along with normal common dallisgrass. The characteristics examined were maturity date, plant height, number of racemes per inflorescence, number of spikelets per raceme, pubescence, stigma and anther color, ergot resistance, seed germination, seed set, pollen stainability, method of reproduction, and chromosome number. There were differences among the regenerants and between them and common dallisgrass for all traits except chromosome number, stigma and anther color, and ergot resistance. One of the more important regenerants had significantly higher seed set than common dallisgrass. All regenerants reproduced by aposporous apomixis but some exhibited a high degree of abortion while others had more aposporous embryo sacs per ovule than common dallisgrass. These findings demonstrate that common dallisgrass can be regenerated through tissue culture and that somaclonal variation is expressed in some of the regenerants, even though some of the altered traits are deleterious.

Introduction

Apomixis is an asexual method of reproduction where seed are produced without fertilization. It is a means whereby plants are cloned through seed, the progeny being uniform and genetically identical to the parent. To successfully use apomixis in an improvement program, it is necessary to have cross-compatible sexual germplasm to use as the female parent to cross with the apomict. When sexual germplasm is not available, essentially the only alternative is to locate naturally occurring superior apomictic ecotypes. However, superior ecotypes or sexual germplasm usually are not available and improvement is not possible. In such cases, less conventional means of producing variation are sometimes attempted. Mutations from ionizing radiation or mutagenic agents are minimally successful. Unfortunately, they usually result in deleterious rather than desired changes. A non-destructive approach is the use of cell and tissue culture technology. Somaclonal variation sometimes occurs in plants regenerated through tissue culture via callus (Orton, 1984; Scowcroft, 1985; Larkin, 1988). This variation

is often perceived as a problem because the regenerants differ significantly from the original genotype, but in apomictic species it could provide a valuable source of genetic variation. The expression of somaclonal variation in apomictic grasses appears to be species dependent. Taliaferro et al. (1989) reported variability in regenerants of two *Bothriochloa* species but Hanna et al. (1984) did not find variability in regenerants of apomictic *Panicum maximum* Jacq.

Common dallisgrass, *Paspalum dilatatum* Poir., is an obligate apomict (Bashaw & Holt, 1958) with 50 chromosomes that associate as 20 bivalents and 10 univalents during meiosis (Bashaw & Forbes, 1958). Because it is meiotically irregular, pollen viability is low and even the viable grains have a variable number of chromosomes. Thus, the grass is not a desirable pollen parent even if a sexual counterpart were available. It is an important forage grass throughout most of the warmer areas of the world but its major limitations are poor seed set and susceptibility to ergot, *Claviceps paspali* Stevens and Hall. Efforts to improve the grass using conventional breeding methods have not been successful because of the absence of sexual reproduction (Burton, 1962). Attempts to induce variation using irradiation were not successful (Bashaw & Hoff, 1962).

Because of this lack of success in creating variation and improving the grass, we regenerated plantlets of common dallisgrass to determine if somaclonal variation is expressed in the regenerated plants.

Materials and methods

Young inflorescences, still enclosed within the leaf sheaths, were collected from field grown common dallisgrass plants and surface sterilized in a 0.5% sodium hypochlorite solution for 10 min followed by 3 rinses in sterile distilled water. The leaves were aseptically removed from the inflorescences, and the inflorescences were dipped into 70% ethanol, cut into 10 to 15 mm segments, and used as explant sources.

For callus formation, pieces of the young inflorescences were placed on 20 ml of medium in 150 ml tubes. The medium contained Murashige & Skoog (1962) (MS) major elements, Schenk & Hildebrandt (1972) minor elements, iron compounds and organics and 9 gl^{-1} Gibco[1] phytagar supplemented with 2.5 mgl^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D). The pH of the medium was adjusted to 5.56 with 0.2 N KOH and it was autoclaved at 138 kPa and 124° C for 30 min. Following autoclaving, filter sterilized thiamine·HCl (5 mgl^{-1}) was added to the medium.

The shoot differentiation medium consisted of the MS medium supplemented with 0.5 mgl^{-1} kinetin, pH 5.8. Filter sterilized thiamine·HCl (5 mgl^{-1}) and 2.5 mgl^{-1} indole-3-acetic acid (IAA) were added after autoclaving. The medium was dispensed as described above.

Cultures containing the initial explants and those used for callus maintenance and increase were grown at 24° C in the dark. Cultures used for shoot differentiation were grown at 24° C, with a 12 h photoperiod at a light intensity of 50 $\mu mol\ m^{-2}\ sec^{-1}$. When the shoots were sufficiently large, root development was obtained by transferring the shoots onto basal MS medium in 125 and 250 ml Erlenmeyer flasks. These flasks were maintained under the same environmental conditions as described for shoot formation. Plantlets with sufficient root growth were transplanted into a soil mixture in small peat pots. These were maintained in a humidity chamber in a greenhouse for about 10 d and then removed from the chamber.

To determine the origin of the young plants, serial sections of the callus were prepared and examined. Pieces of callus at different developmental stages were fixed in FAA, dehydrated in the tertiary butyl alcohol series, embedded in paraffin, sectioned at a thickness of 15 µ, stained with the safranin 0-fast green series, and examined with light microscopy.

A total of 682 regenerated plantlets were planted into peat pots in the greenhouse. Four hundred and one of these survived and were transplanted into a

[1] Mention of a trademark of proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

field nursery on 1 m centers in a Houston black clay soil (fine montmorillonitic, thermic Udic Pellusterts). Based on morphological characteristics and preliminary heat tolerance data, 45 regenerants were selected for further study. Each regenerant was separated into 18 clonal plants in the greenhouse. In late March 1985, these 45 regenerants and common dallisgrass that had not been used in the tissue culture program were established in a nursery with 6 replications planted in a randomized complete block design. A replication consisted of three clones of each entry.

Plant maturity was evaluated by visually ranking the amount of floral initiation for each entry on June 20, 1985 and May 21, 1986. The scale ranged from 1 to 5, with 5 denoting the presence of mature inflorescences (indistinguishable from common dallisgrass), and 1 indicating no visible inflorescences. Based on these findings, 24 entries were considered identical to common dallisgrass, and the remaining 21 entries were different. Of these, three lacked persistence and were eliminated from the study. Although entries 656 and 282 had similar maturity scores to common dallisgrass, their culm angle and diameter differed, and both were included in the study.

In 1986 common dallisgrass and these 20 entries were studied more extensively. Plant height was determined by measuring the distance of both the tallest leaf and inflorescence from their tips to the soil surface. The number of racemes per inflorescence and spikelets per raceme were counted. Pubescence was determined by visually examining the plants. Seed germination was determined by counting 500 florets at random from the bulked florets harvested from two to four open-pollinated inflorescences of each entry and these were placed into five Petri dishes (100 florets/dish) which were maintained in a Stultz[1] germinator (12 h photoperiod; 12 h 35° C/12 h 25° C) for 21 days). The number of seedlings emerging from the florets during the 21 day period were counted and the per cent germination was calculated. In dallisgrass the caryopsis is enclosed by an indurate lemma and palea of the floret and because of this it is difficult to determine if a caryopsis is present in a mature floret. The same florets used for the germination study were used to determine seed set. After the germination tests were completed, the remaining non-germinated florets in each Petri dish were dissected open and examined under a stereozoom-microscope for endosperm development. Seed set was determined by adding the number of germinated florets to the number of ungerminated florets that contained endosperm and calculating the per cent seed set. Ergot susceptibility and stigma and anther color were determined by visually examining the florets. Pollen viability was estimated by observing a minimum of 500 pollen grains per plant stained in potassium-iodine (I_2-KI). Method of reproduction was determined by cytologically examining embryo sac development in the ovules. Inflorescences were collected and fixed in FAA. The pistils were dissected from the florets, dehydrated and prepared by one of two methods. Some were cleared with methyl salicylate and observed using interference microscopy (Young et al., 1979). Others were embedded in paraffin, sectioned at a thickness of 15 μ, stained with safranin 0-fast green and examined with light microscopy. Chromosome number was determined primarily for those plants with abnormal floral development. Root tip cells were examined to determine their chromosome number using a technique described elsewhere (Burson, 1991).

Seed from 11 of the regenerants and common dallisgrass were germinated and used to establish a nursery of the SC_2 progeny. It consisted of 6 replications with 24 progeny of each entry in each replication. These plants were observed to determine if the variable morphological traits were expressed.

Analysis of variance and Duncan's Multiple Range test were used to analyze and rank the various data sets of the 20 regenerants and common dallisgrass. However, the more conservative Dunnet's t test was used to compare common dallisgrass to the 20 regenerants for the traits quantified. Appropriate data transformations (square root for germination data, arcsin $\sqrt{\%}$ for seed set data) were applied to satisfy assumptions of the analysis of variance (Steel & Torrie, 1980), although the actual values are present in Table 4.

Results and discussion

Callus formation and plantlet regeneration

Callus first appeared in about 10 to 14 days. It formed on the rachis, pedicels, and spikelets of the immature inflorescences. Three types of callus were noted. The most prevalent type was friable and yellow-gray. Another type was less friable and white. The third and least common was of a gelatinous nature that developed primarily on the glume and lemma of the young spikelets and florets. After the callus increased in quantity, the yellow-gray friable type was sub-cultured one to three times for periods of 4 to 6 weeks before transfer to the shoot differentiating medium.

The first visible evidence of shoot development occurred in about 10 days. Most shoots were green but there was an occasional albino. Anatomical examination of the callus tissue revealed that differentiation was monopolar or bipolar indicating the plantlets developed through both organogenesis and embryogenesis. Monopolar development was more common. Mixed cultures of this nature have been observed in other grass species. Fladung & Hesselback (1986) reported similar findings to ours in *Panicum bisulcatum* Thumb., although Akashi & Adachi (1992) and Davies et al. (1986) reported that dallisgrass regenerated only by somatic embryoge-

Table 1. Maturity rankings of common dallisgrass and tissue culture-derived dallisgrass regenerants (June 20, 1985 and May 21, 1986)

Entry	1985	1986
Com	5.00[1]a[2]	5.00 a
100	5.00 a	4.83 ab
325	5.00 a	4.83 ab
332	5.00 a	4.83 ab
309	5.00 a	4.83 ab
170	5.00 a	4.83 ab
190	5.00 a	4.83 ab
594	5.00 a	4.83 ab
460	5.00 a	4.33 bc
323	4.17 c	4.17 c
143	4.83 a	3.50 c
491	4.33 bc	3.00 e
195	4.67 ab	2.67 e
376	2.67 e	1.83 f
107	2.67 e	1.67 f
129	3.50 d	1.50 fg
377	1.83 f	1.50 fg
605	1.17 g	1.33 fg
150	1.00 g	1.00 g
258	1.33 g	1.00 g
257	1.00 g	1.00 g

[1] Scoring based on 5 point system where 5 is equivalent to common, with mature seed present, and 1 indicates no seedheads visible.

[2] Values in a column not followed by the same letter are significantly different, Duncan's Multiple Range Test (P = 0.01%).

Table 2. Values for different characteristics of common dallisgrass and tissue culture-derived dallisgrass regenerants

Characteristic	Minimum	Maximum	Mean	Common	Dunnet's t	No. regenerants different from common dallisgrass
Maturity 1985	1.0	5.0	4.4	5.0	1.15**	8
Maturity 1986	1.0	5.0	4.3	5.0	1.28**	11
Leaf height (cm)	48.2	70.8	60.0	61.7	12.3*	1
Inflor. height (cm)	73.7	175.7	126.7	146.0	34.2**	8
Racemes/Inflor. (no.)	3.3	5.0	4.2	4.7	1.3*	1
Spikelets/Raceme (no.)	35.4	98.9	71.8	96.4	34.6**	8
Seed germination (%)	0.0	8.0	2.9	2.2	4.2**	1
Seed set (%)	0.0	36.2	15.1	20.6	12.4**	9

* Significant at the 0.05% level of probability.

** Significant at the 0.01% level of probability.

nesis. A possible reason for this difference may the different growth regulators used.

Morphological characteristics

The entries varied for maturity date (Table 1) and some matured significantly later than common dallisgrass (Table 2). Maturity date for most entries was similar from one year to another (Table 1). Correlation coefficients between years were highly significant ($p = 0.0001$) when values of 5.0 were excluded, and common dallisgrass was either included ($r = 0.88$) or excluded ($r = 0.74$) from the analysis. However, the entry × year interaction was significant ($p = 0.0001$) and the data could not be pooled across years. Some later maturing entries had poorly developed spikelets and inflorescences which may be associated with deleterious physiological changes in these regenerants.

One of the most obvious differences among the regenerants was plant size, especially height. There was a wide range among the regenerants for leaf and inflorescence height (Table 3). None of the regenerants were significantly taller than common dallisgrass but eight were significantly shorter (Table 2). Entry 257 was the smallest regenerant (Table 3), and although there were differences among the regenerants in leaf height, only entry 257 had significantly lower leaf height than common dallisgrass.

There was considerable variation in inflorescence morphology (Table 3). Dunnet's test indicated there was a significant difference between common dallisgrass and only one entry (605) for the number of racemes per inflorescence (Table 2). All inflorescences of entries 257 and 491 had 5 racemes. However, racemes of 257 were reduced to rudimen-

Table 3. Morphological characteristics of common dallisgrass and tissue culture-derived dallisgrass regenerants

Entry	Plant height		Inflorescences	
	Inflorescence height (cm)	Leaf height (cm)	Racemes/inflor. (no.)	Spikelets/raceme (no.)
656	175.7 a	63.2 a–d	4.00 a	96.7 a
282	161.8 ab	57.0 a–d	4.00 a	96.2 a
325	158.2 a–c	58.2 a–d	4.17 a	93.9 a
309	150.3 bc	52.3 cd	3.67 a	94.2 a
594	150.0 bc	68.3 a–c	4.83 a	86.3 a–c
Com	146.0 bc	61.7 a–d	4.67 a	96.4 a
332	145.2 bc	53.7 b–d	4.00 a	91.8 a
170	142.3 b–d	55.5 a–d	4.17 a	98.9 a
323	141.4 b–d	63.2 a–d	4.00 a	67.5 c–f
100	139.3 cd	60.5 a–d	4.83 a	89.4 ab
460	139.2 cd	54.7 a–d	3.67 a	70.2 b–e
195	123.2 de	61.2 a–d	4.00 a	78.8 a–d
491	122.8 de	70.8 a	5.00 a	57.0 e–g
377	111.2 ef	59.5 a–d	4.20 a	44.7 g–i
129	106.8 ef	70.3 ab	4.33 a	39.6 hi
376	103.8 ef	63.2 a–d	3.83 a	63.4 d–g
258	99.0 fg	62.7 a–d	4.33 a	41.9 hi
107	97.0 fg	57.5 a–d	4.83 a	47.8 f–h
150	93.8 fg	59.0 a–d	5.00 a	43.3 [2]
605	81.0 gh	59.3 a–d	3.33 a	35.4 i
257	73.7 h	48.2 d	5.00 a	35.4 i

[1] Values in a column not followed by the same letter are significantly different, Duncan's Multiple Range test.

[2] Data for this entry was taken from only one replication and it was not used in the statistical analysis.

tary forms and were much smaller and less developed than those of the other regenerants.

The number of spikelets per raceme also varied (Table 3). Most regenerates had fewer spikelets than common dallisgrass and eight entries had significantly less (Table 2). Three entries, 150, 605, and 257, did not have fully developed spikelets at maturity. This influenced other characteristics, especially seed set (Table 4).

The most striking change in the regenerants was the amount of pubescence on the leaves and stems. There were varying degrees of pubescence among the regenerants but entry 100 was very pubescent. SC_2 and SC_3 progeny from this regenerant were grown in a field nursery and the trait persisted. This trait may be a mutation of a single gene in common dallisgrass. Owen (1966) reported a naturally occurring pubescent common dallisgrass plant that apparently resulted from a point mutation. The pubescent somaclone appeared very similar to the mutant described by Owen. Because common dallisgrass has one genome in the haploid condition, the mutation of an allele on any of these univalents, regardless of whether recessive or dominant, will be expressed. It is possible that pubescence is controlled by a single gene located on one of the univalents, and this gene may have mutated to produce the pubescent somaclone.

The sticky 'honey dew' exudate was present on the inflorescences of all regenerants, indicating susceptibility to ergot. There was less honey dew on the florets of the entries with poorly developed inflorescences. All 401 regenerants in the original field nursery had purple anthers and stigmas, the same as common dallisgrass.

All SC_2 progenies from 11 of the regenerants were examined and they maintained the same observable morphological traits that were expressed in their SC_1 parents. This indicates the variability expressed in the original regenerants is somaclonal variation and is not of an epigenetic nature.

The only other report of somaclonal variation in dallisgrass was by Davies et al. (1986). They reported several regenerants with some altered morphological traits. However, their data was collected from single plants that were not replicated and the SC_2 generation was not evaluated.

Cytological and fertility characteristics

Because aneuploidy might be the cause for poor spikelet and floret development, the somatic chromosome number was determined for those entries with poorly developed inflorescences and also for five entries with normal inflorescences. All had 50 chromosomes, as did common dallisgrass.

Pollen stainability ranged from 0 to 57% and was 23% for common dallisgrass (Table 4). Entries with little or no stainable pollen had poorly developed spikelets and florets. Surprisingly, entry 257 produced a few viable pollen grains (0.6%). Stainability in five entries was higher than that of common dallisgrass and two (605 and 100) were considerably higher (Table 4).

Seed from the regenerants and common dallisgrass germinated poorly (0 to 8.0%; Table 4), which

Table 4. Seed set and germination and pollen stainability in common dallisgrass and tissue culture-derived dallisgrass regenerants

Entry	% Seed set	% Seed germination	% Pollen stainability
309	36.2 a[1]	5.0 a–c	17.6
332	31.4 ab	3.0 b–d	17.3
325	30.0 a–c	8.0 a	23.7
282	28.2 a–c	3.6 b–c	6.0
170	26.6 a–c	2.6 b–d	24.9
656	25.6 bc	4.4 a–c	29.6
100	21.2 c	5.4 ab	57.5
Com	20.6 c	2.2 c–e	23.4
594	11.2 d	0.4 ef	4.3
460	11.2 d	2.8 b–d	16.8
491	5.6 de	1.0 d–f	15.3
195	5.2 e	0.6 f	19.4
323	5.0 e	1.0 ef	7.9
129	1.5 f	1.0 ef	9.1
376	0.6 f	0 f	0[3]
377	0.4 f	0 f	0[3]
258	0.2 f	0 f	5.6
257	0 f	0 f	0.6
605	0 f	0 f	43.5
107	0 f	0 f	8.9
150	NA[2]	NA	12.6

[1] Values in a column not followed by the same letter are significantly different, Duncan's Multiple Range Test.

[2] Entry 150 did not produce viable seed.

[3] Entries 376 and 377 did not produce viable pollen.

could be due to dormancy. Germination of one entry (325) was significantly higher than that of common dallisgrass (Table 2). Seed set is probably the most important trait evaluated because it is a major impediment of common dallisgrass. There was a wide range for seed set among the regenerants (Table 4), and the value for common is near its maximum under normal conditions. Seed set of nine regenerants was significantly different from common (Table 2). However, only one (309) was significantly higher. This entry and possibly some of the more fertile regenerants in Table 4 are probably the most important somaclones recovered. Sterility in entries 150, 257, and 605 is undoubtedly in part due to their poor spikelet development.

Across all entries, pollen stainability and seed set were positively correlated ($p = 0.05$). All entries with higher seed set produced some stainable pollen; whereas, those producing little or no seed, with one exception, had very little or no stainable pollen. However, those entries with the most stainable pollen were not the most fertile. Since these plants reproduce by apomixis and are pseudogamous, these findings are not surprising because only a limited quantity of viable pollen is necessary for endosperm development.

Mature ovules of 20 regenerants and common dallisgrass were examined cytologically. All embryo sacs observed were aposporous apomictic sacs similar to those in common dallisgrass. Most mature sacs contained an egg cell and two polar nuclei. The number of sacs per ovule ranged from 1 to 10, but most had from one to three (Table 5). The average number of sacs per ovule ranged from 1.43 to 4.39 for the regenerants and was 3.87 for common dallisgrass (Table 5). When ovule abortion is not a factor, it appears the more fertile entries have the greater number of sacs per ovule. Entry 309 was the most fertile and had the highest frequency of sacs. There was considerable deterioration of the embryo sacs in several entries with entry 257 being the highest (72.7%; Table 5). Most of these regenerants had poor floret development and consequently low seed set. These findings indicate that regeneration

Table 5. Embryo sacs in mature ovules of common dallisgrass and the tissue culture-derived dallisgrass regenerants

Entry	No. ovules obs.	No. aposporous embryo sacs/ovule		Ovules with deteriorated sacs	
		Range	Ave./ovule	No.	%
100	18	1–5	2.72	0	0
107	16	1–5	3.10	6	37.5
129	31	1–10	3.60	11	35.5
150	15	1–3	2.09	4	26.7
170	18	1–7	4.21	4	22.2
195	28	1–8	3.44	1	3.6
257	44	1–3	1.58	32	72.7
258	13	1–3	1.69	0	0
282	11	1–4	2.64	0	0
309	36	2–7	4.39	0	0
323	57	1–8	3.26	0	0
325	14	1–3	1.80	9	64.3
332	25	1–6	3.00	0	0
376	11	1–3	1.43	4	36.4
377	34	1–6	2.10	4	11.8
460	34	1–5	2.76	1	2.9
491	38	1–6	3.58	0	0
594	25	1–7	2.92	0	0
605	55	1–6	3.10	4	7.3
656	13	1–6	3.38	0	0
Com	31	1–9	3.87	0	0

did not alter the reproductive behavior of the regenerants except there was more deterioration of the reproductive tissue in some plants and others consistently had more sacs than common dallisgrass. Deterioration was probably due in part to the poor floral development but there were some entries with a relatively high deterioration that had well developed florets.

Findings from this study demonstrate that common dallisgrass can be regenerated through tissue culture and somaclonal variation is expressed in some of the regenerants. This variation was fixed in the plants for future generations because the plants continued to reproduce by apomixis. For most characteristics examined, there were variants that differed from common dallisgrass. Most altered traits were deleterious but the regeneration with increased seed set may well be valuable since low seed set is a major problem in common dallisgrass. Thus, the results suggest that tissue culture is a useful tool for improving obligate apomicts such as common dallisgrass.

References

Akashi, R. & T. Adachi, 1992. Somatic embryogenesis and plant regeneration from cultured immature inflorescences of apomictic dallisgrass (*Paspalum dilatatum* Poir.). Plant Sci. 82: 213–218.

Bashaw, E.C. & I. Forbes, Jr., 1958. Chromosome numbers and microsporogenesis in dallisgrass, *Paspalum dilatatum* Poir. Agron. J. 50: 441–445.

Bashaw, E.C. & B.J. Hoff, 1962. Effects of irradiation on apomictic common dallisgrass. Crop Sci. 2: 501–504.

Bashaw, E.C. & E.C. Holt, 1958. Megasporogenesis, embryo sac development and embryogenesis in dallisgrass, *Paspalum dilatatum* Poir. Agron. J. 50: 753–756.

Burson, B.L., 1991. Homology of chromosomes of the X genomes in common and Uruguayan dallisgrass, *Paspalum dilatatum*. Genome 34: 950–953.

Burton, G.W., 1962. Conventional breeding of dallisgrass, *Paspalum dilatatum* Poir. Crop Sci. 2: 491–494.

Davies, L.J., D. Cohen, K.V. Mullins & S.S. Bhojwani, 1986. Somaclonal variation in the apomictic pasture grass *Paspalum dilatatum*. In: T.A. Williams & G.S. Wratt (Eds). Proc. DSIR Plant Breeding Symp., New Zealand Agron. Soc. Spec. Publ. No. 5, p. 355–360.

Fladung, M. & J. Hesselbach, 1986. Callus induction and plant regeneration in *Panicum bisulcatum* and *P. miliodes*. Plant Cell Rep. 3: 169–173.

Hanna, W.W., C. Lu & I.K. Vasil, 1984. Uniformity of plants regenerated from somatic embryos of *Panicum maximum* Jacq. (Guinea grass). Theor. Appl. Genet. 67: 155–159.

Larkin, P.J., 1988. Somaclonal variation: history, method, and meaning. Iowa State J. Res. 62: 571–585.

Murashige, T. & F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physio. Plant. 15: 473–497.

Orton, T.J., 1984. Somaclonal variation: Theoretical and practical considerations. In: J.P. Gustafson (Ed). Genetic Manipulation in Plant Improvement, 16th Stadler Genet. Symp., p. 427–468. Plenum Press.

Owen, C.R., 1966. A hirsute ecotype isolated from common dallisgrass (*Paspalum dilatatum* Poir.). Crop Sci. 6: 374–375.

Schenk, R.U. & A.C. Hildebrandt, 1972. Medium and techniques for induction of growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50: 199–204.

Scowcroft, W.R., 1985. Somaclonal variation: the myth of clonal uniformity. In: B. Hohn & E.S. Dennis (Eds). Genetic Flux in Plants, p. 217–245. Springer Verlag.

Steel, R.G.D. & J.H. Torrie, 1980. Principles and Procedures of Statistics. 2nd ed. McGraw Hill Book Co., New York.

Taliaferro, C.M., S.M. Dabo, E.D. Mitchell, B.B. Johnson & B.D. Metzinger, 1989. Morphologic, cytogenetic, and enzymatic variation in tissue culture regenerated plants of apomictic old-world bluestem grasses (*Bothriochloa* sp.). Plant Cell, Tiss. Org. Cult. 19: 257–266.

Young, B.A., R.T. Sherwood & E.C. Bashaw, 1979. Cleared-pistil and thick-sectioning techniques for detecting aposporous apomixis in grasses. Can. J. Bot. 57: 1668–1672.